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Proceedings

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IN TOMORROW'S WHEY INDUSTRY

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In recent years, no industry has been regulated more extensively by the Federal government than the food industry. In many cases in the past, because a substance to be regulated did not fall neatly into the domain of any one Federal agency, separate approaches were taken to regulate it. This frequently resulted in an unworkable situation that caused serious problems, both for the agencies and for the industry.

E. J. Allera of the U.S. Food and Drug Administration pointed out that, precisely because of the separate but equal system of operation among the agencies in their various spheres, two of the major issues in the future of food regulation have to be ecumenism and efficiency (1). It is in the interest of everyone that the most efficient regulatory scheme be carried out to facilitate discussion and ultimate resolution of the issue in question. Coordination of effort among the regulatory agencies regarding a specific compound is absolutely necessary if the regulatory process is to be accelerated and made more efficient. Before there can be any regulation, there must be research and analysis to determine if a given substance is harmful (and, if so, in what quantity) and then how to detect it in order to enforce compliance.

The U.S. Department of Agriculture has made a contribution toward ecumenism through a memorandum of understanding relative to cooperation with respect to research. This was signed in April 1980 between the Science and Education Administration (SEA), of which Agricultural Research is a part, and the Food Safety and Quality Service (FSQS). Each year, FSQS is to provide SEA with documents describing new research needs for assuring the safety and quality of inspected and graded foods at the lowest cost to the public. Information is to be provided on each project, including the objective of the work, why the work is needed, and its priority relative to other projects requested or planned. SEA has the responsibility to provide FSQS with summaries of research being planned or conducted that may have an impact on FSQS. Each year, a joint technical review will be made of research in progress, new research requested by FSQS, other SEA research that could meet some FSQS goals and a complete exchange of technical information will be maintained.

FSQS RESEARCH NEEDS

At present, the FSQS has formally identified two research needs in the dairy area: 1) to determine pasteurization parameters for cheese wheys and high acid products and 2) to determine pasteurization time/temperature requirements for low fat, unsweetened dairy products with increased milk

solids or total solids higher than fluid milk. In addition, the FSQS, the Whey Products Institute (WPI), and industry sources have informally identified the need for a new scorched particle test applicable to acid whey powder.

In response to a formal FSQS request, ERRC's Food Science Laboratory personnel have developed a detailed work plan covering both pasteurization requirements. It would be advantageous to industry if lowered time/temperature pasteurization parameters could be established for high acid products such as cottage cheese whey and still provide recognized public health assurance. The establishment of minimum heating standards is a consideration, not only for health protection but also for reducing protein denaturation and energy usage.

Because of the development of new processing technologies, FSQS is concerned that existing time/temperature pasteurization standards, especially minimum high temperature-short time (HTST) pasteurization at 71.7 C for 15 seconds, may not suffice for such products as cheese milks fortified with nonfat dry milk, membrane processed milks and wheys for cheese making and other food uses, and wet blended or extruded products containing dairy ingredients.

PASTEURIZATION EFFECTS ON WHEYS

One preliminary experiment has been conducted at ERRC on the effects of HTST heat treatment at different temperatures on the kill of Staphylococcus aureus inoculated into fluid wheys of varying pH (2). A laboratory scale tubular heater with a flow of 180 ml/minute was used for the heat treatments. The data, listed in Table I, show that, as might be expected, the greatest kill occurred in the whey with the most acid pH, 4.52; the kill was over 90 percent, even at 63 C. At temperatures of 69 and 72 C, the kill of S. aureus was over 99 percent, regardless of whey pH. Heat treatment at 72 C for 15 seconds is the minimum pasteurization standard for whey at present (3). S. aureus was chosen as the test organism because staphylococci are the organisms most commonly involved in dairy product associated food poisonings (4) and staphylococcal contamination can be a particular problem during cheese making (5).

The effects of heat treatments on whey protein denaturation have been studied in much greater detail. Cottage cheese whey proteins were more resistant than similar proteins in skimmilk to heat denaturation during prolonged heating (6). Shifting the pH toward alkalinity before heating at 82 C for 30 minutes increased the rate of protein denaturation; minimum denaturation was observed at pH 3.4 (Figure 1). A modified Harland-Ashworth procedure was used for measurement of protein denaturation (7).

No simple effect on denaturation was observed on increasing the total solids of the whey before heating for 30 minutes at different temperatures (Figure 2). Although increasing the temperature of heating from 74 to 87 C increased the amount of protein insolubilized, maximum protein stability was observed at approximately 20 percent total solids in all cases. The reason for the observed stability has never been identified, but it could be related to the aggregation behavior of β -lactoglobulin, the major whey protein, upon heating.

TABLE I.--Staphylococcus aureus kill in fluid wheys given HTST heat treatments at varying temperatures for 15 seconds

		рН		
Heating	6.42	4.60	4.52	~5.8 ^a
temperature C		% kil	.led	
63	56.3	52.6	92.6	55.0
66	99.8	98.3	99.9	92.5
69	99.8	99.8	100	99.7
72	99.8	100	100	99.2

a Prepared by mixing sweet and acid wheys.

THE EFFECT OF PH ON WHEY PROTEIN DENATURATION

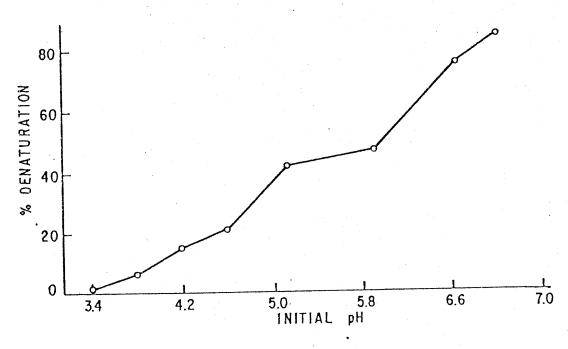


Figure 1.—Effect of pH adjustment before heating at 82 C for 30 minutes on denaturation of cottage cheese whey proteins as measured by a modified Harland-Ashworth procedure.

EFFECT OF TOTAL SOLIDS ON WHEY PROTEIN DENATURATION

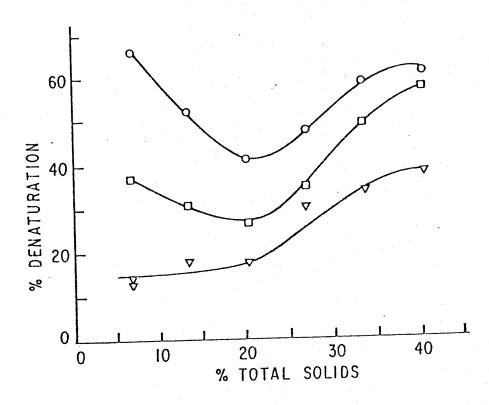


Figure 2.—Effect of total solids concentration on denaturation of cottage cheese whey proteins when heated at different temperatures for 30 minutes.

0 = 87 C□ = 84-5 C ∇ = 74 C

The effects of pasteurization on protein denaturation were not investigated in the above study; the times and temperatures examined were all considerably above high temperature-short time (HTST) and vat pasteurization standards. Therefore, a series of experiments was conducted to determine the effects of HTST pasteurization at $165\ F\ (73.9\ C)$ for $20\ seconds$ with a Mallory tubular heater on whey protein solubility at three different protein concentrations. The pasteurization time and temperature selected were higher than the minimum pasteurization requirement of 161 F (71.7 C) for 15 seconds (8) because of equipment limitations.

The wheys to be studied were first clarified and/or defatted by passing them through a cream separator before further processing. The percentage of protein nitrogen insolubilized by the heat treatment was measured at pH 5.1 by the Kjeldahl procedure of Rowland (9); heat denatured whey proteins were removed from the sample after precipitation in acetate buffer at pH 5.1, and the nitrogen content of the filtrate was measured by a Kjeldahl procedure.

The undenatured whey protein nitrogen is the fraction precipitated not by acetate but by 12 percent trichloroacetic acid. Whey protein concentrates were prepared by fractionating the defatted clarified whey in an Abcor pilot scale 44S batch ultrafiltration (UF) unit. This unit contained 4.09 sq. m of 3.05 m long HFA membranes with a rated retention value of 98 percent for whey proteins. Concentrates containing very high levels of protein were prepared by preconcentrating at 48.9 C by UF at an inlet pressure of 3.16 kg/cm and an outlet pressure of 1.05 kg/cm. The protein was separated further from low molecular weight material by eluting the UF retentate with deionized water at 25 C and a flow rate of 4 l/min from a Pharmacia Model GF 04-10 pilot scale Sephadex column containing G25 coarse Sephadex beads.

HTST pasteurization at 73.9 C for 20 seconds had no effect on the solubility of cheddar cheese whey proteins at pH 6.4 (Table II); the protein fraction comprised 12.6 percent of the total solids. Concentration of the protein by UF to 55.8 percent of the total solids (16.4 percent) increased insoluble material slightly; upon HTST pasteurization, insoluble material considerably increased. Elution of the unpasteurized UF retentate from the Sephadex column reduced the total solids concentration to 4.94 percent and increased the protein concentration to 82.9 percent of the solids; a slight increase in insoluble protein was also measured. Upon pasteurization, a sharp increase in insoluble material occurred, greater than that observed when the UF retentate was pasteurized. These data show that removal of the low molecular weight material resulted in severe heat damage to the proteins even when subjected to the lowest HTST pasteurization possible with the equipment available.

TABLE II.--Solubility of cheddar cheese whey proteins pasteurized at 165 F (73.9 C) for 20 seconds.

		ration
12.6	55.8	82.9
•		
15.7	18.1	21.0
14.3	28.8	37.8
6.77	16.4	4.94
	12.6 % of prinsolution 15.7 14.3	% of protein nitro insoluble at pH 5 15.7 18.1 14.3 28.8

HTST pasteurization at pH 4.7 caused similar heat damage to the proteins of clarified cottage cheese whey and the UF retentate and gel column eluate prepared from it (Table III). The amount of insoluble protein measured after pasteurization was less in all cases than that found with cheddar whey. Insoluble material in the raw fluid cottage cheese whey was quite low (<2 percent) but, in contrast to the cheddar whey, increased sharply upon pasteurization.

TABLE III. -- Solubility of cottage cheese whey proteins pasteurized at 165 F (73.9 C) for 20 seconds

	Crude prot	ein concen of solids	tration
	11.0	45.4	77.6
	% of Pr insolu	rotein nitr uble at pH	ogen 5.1
Raw	1.8	9.2	8.6
Pasteurized	10.6	24.0	26.1
73.9 C, 20 sec.			
Total solids pasteurized (%)	6.44	12.5	3.9

Because HTST pasteurization of whey protein concentrates at pH 6.4 and pH 4.7 destabilized large amounts of protein and because the work of Guy et al. (6) had shown that destabilization of cottage cheese whey proteins by heat was minimal at pH 3.5, the effects of HTST pasteurization on protein solubility at this pH were investigated in an attempt to reduce the quantity of material insolubilized. Accordingly, fresh fluid cottage cheese whey was brought to pH 3.5 with hydrochloric acid and subjected to ultrafiltration and gel permeation as previously described. Contrary to expectations, denaturation of the raw whey upon pasteurization was just as great as for pasteurization of the whey at pH 4.7 (Table IV). In addition, UF at pH 3.5 seems to have brought about a much greater insolubilization of the proteins at pH 3.5 than at the other two pH's investigated. These results are probably typical of the recirculating batch UF process used and would most likely not occur in a continuous process. However, subsequent HTST pasteurization of either the UF retentate or the gel column eluate prepared from it did not significantly increase the amount of protein insolubilized.

Because vat pasteurization of whey at $145~\mathrm{F}$ (62.8 C) for 30 minutes is also carried out in some processing operations, its effect on the solubility of cheese whey proteins was also investigated. Vat pasteurization produced a greater protein insolubilization of cottage cheese whey protein at pH 4.7

(Table V) than did HTST pasteurization (Table III). Greatest insolubilization occurred in the whey protein concentrates.

TABLE IV.--Solubility of cottage cheese whey proteins adjusted to pH 3.5 and pasteurized at 165 F (73.9 C) for 20 seconds

		cein concer of solids	ntration
	11.0	44.5	69.9
		rotein nit uble at pH	
Raw	1.32	34.6	31.8
Pasteurized 73.9°C, 20 sec.	13.2	35.0	32.7
Total solids pasteurized (%)	6.30	11.8	2.3

TABLE V.--Solubility of cottage cheese whey proteins vat pasteurized at 145 F (62.8 C) for 30 minutes

de la companya de la		tein concent	ration
	11.0	45.9	85.5
	% of Protein nitrogen insoluble at pH 5.1		
Raw	4.88	7.8	7.62
Pasteurized 62.8 C, 30 min.	16.3	31.7	27.6
Total solids pasteurized (%)	6.43	12.2	3.18

(Table V) than did HTST pasteurization (Table III). Greatest insolubilization occurred in the whey protein concentrates.

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TABLE V.--Solubility of cottage cheese whey proteins vat pasteurized at 145 F (62.8 C) for 30 minutes

Crude protein concentration % of solids			
11.0	45.9	85.5	
4.88	7.8	7.62	
16.3	31.7	27.6	
6.43	12.2	3.18	
	% of Prinsolu 4.88	% of solids 11.0 45.9 % of Protein nitro insoluble at pH 5 4.88 7.8 16.3 31.7	

Because it was possible that turbulence in the line during the heat treatment could contribute to protein denaturation as the wheys, especially the whey protein concentrates, were pumped through the tubular heater, HTST neating and holding times were simulated under still conditions with a microwave oven. Although come-up times varied with the temperatures, holding times were 20 seconds in all cases. Insolubilization of the protein in a gel column eluate isolated from cottage cheese whey and containing 3.2 percent total solids, 81 percent of which was crude protein, increased logarithmically with the increase in heating temperature (Figure 3). At 73.9 C, about 28 percent of the protein nitrogen was insolubilized; this value compared quite well with the 26 percent of insoluble material measured when the gel column eluate was pasteurized in the tubular heater (Table III). From these data, under the experimental conditions used, turbulence did not appear to contribute to protein denaturation even in whey protein concentrates containing minimum amounts of low molecular weight material.

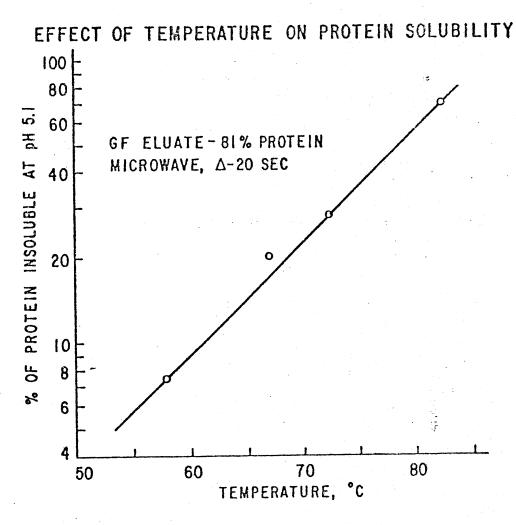


Figure 3.—Effect of heating temperature on protein denaturation of a gel column eluate containing 3.2 percent total solids, 81 percent of which was crude protein. Holding times were 20 seconds in all cases.

A UF retentate from cottage cheese whey, containing 44.7 percent crude protein on a solids basis, was condensed in vacuo in a Harris-Weigand falling film evaporator under minimum heating conditions of 60-60 C to 36 percent total solids. A series of dilutions was then made from the condensed material in order to investigate the effect of varying total solids levels on protein denaturation during HTST heat treatment. Because of the possibility that serious burn-on and gelling of the samples containing higher total solids serious burn-on and gelling of the samples containing higher total solids levels could occur in the tubular heater, heating was carried out in the microwave oven. Even here, 67 C was the maximum temperature found possible for heating because serious problems with localized heating and gelling occurred at pasteurization temperatures. At a crude protein concentration of 44.7 percent of the solids, variation in the total solids proved to have little effect on the amount of protein insolubilized once the total solids level exceeded about 8 percent (Figure 4).

EFFECT OF TOTAL SOLIDS ON PROTEIN SOLUBILITY

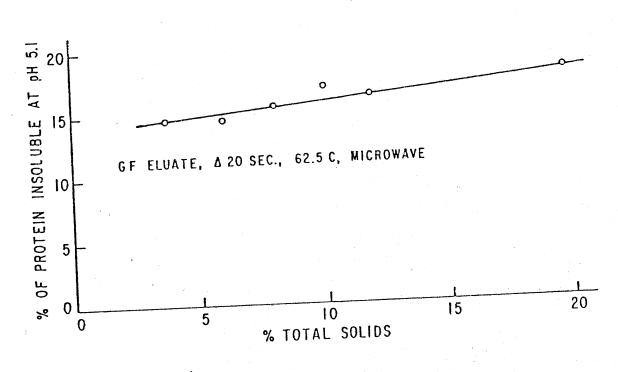


Figure 4.—Effect of total solids concentration on protein denaturation of an ultrafiltration retentate containing 44.7 percent crude protein. Heating temperature was 67 C and holding time 20 seconds in all cases.

A similar experiment was carried out with a gel column eluate containing 84 percent crude protein on a solids basis and condensed to 25 percent total solids. The high viscosities encountered precluded condensing to higher total solids levels. The heating temperature had to be reduced still further, to 62.5 C, to avoid gelling at the higher solids levels. At this protein

concentration and heating temperature, a slow increase in protein insolubilization occurred with increaing total solids concentration (Figure 5).

EFFECT OF TOTAL SOLIDS ON PROTEIN SOLUBILITY

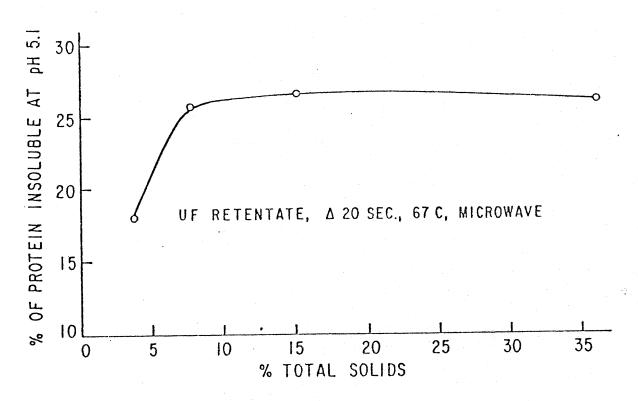


Figure 5.—Effect of total solids concentration on protein denaturation of a gel column eluate containing 84 percent crude protein. Heating temperature was 62.5 C and holding time 20 seconds in all cases.

These experimental results suggest that, at least in the case of whey protein concentrates, the total solids concentration pasteurized by HTST procedures may be limited by the point at which viscosity buildup and sample gelation occur. In an ultrafiltered whey protein concentrate containing 30 percent total solids, viscosity buildup began at about 57 C (Figure 6) followed almost immediately by gelation. In contrast, a gel column eluate containing 14 percent total solids, showed no significant increase in viscosity with temperature until about 77 C; at total solids levels higher than 14 percent gelation occurred at about 50 C.

On the basis of these results, therefore, the initial evaluations of HTST pasteurization parameters will be carried out on fluid and condensed wheys rather than whey protein concentrates.

SCORCHED PARTICLES

During food powder manufacture, a certain amount of scorching occurs on some of the hot surfaces of the dryer, and the particles inevitably find

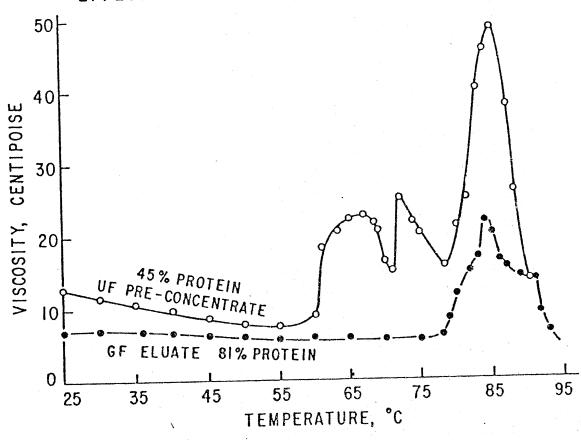


Figure 6.—Effect of heat on viscosity of an ultrafiltration retentate containing 30 percent total solids and viscosity of a gel column eluate containing 14 percent total solids.

their way into the powder. At present, the scorched particle content of whey powders is determined by visual comparison of the contents of a standard sediment disc with the U.S. Scorched Particle Standard for Dry Milks (10). Although this standard procedure works very well with sweet wheys, FSQS personnel have found that acid wheys, especially high heat powders, will not filter through the sediment disc upon reconstitution (11). FSQS has made allowances for this in inspection and grading procedures with the proviso that, when a new scorched particle test is developed and field tested, acid whey powder will also be required to meet minimum standards for scorched particles.

The most promising approach to a new test appears to be the resolubilization of heat denatured whey proteins by partial enzymatic hydrolysis. It has been reported (12) that complete protein solubility may be obtained by treatment with trypsin at either neutral pH or pH 3.5. Unfortunately, minimum solubility was found to be at pH 4.7, the pH of cottage cheese whey, so some pH adjustment would be necessary. Papain may be a more appropriate enzyme to use for this purpose, since its point of maximum activity is

around pH 4.0. Time constraints may be a problem with this procedure because a two hour digestion period is necessary. Additional work is needed to stablish the experimental conditions necessary to produce a filterable sample in the least possible time.

LACTOSE ANALYSIS

The Whey Products Institute has identified several research areas for the 1980's considered to be worthwhile. These include anaerobic whey fermentation for producing methane gas as an alternate fuel source, energy conservation techniques in whey processing, applications of modified whey products as food ingredients, recovery and use of salt drippings to meet EPA standards regarding their disposal, methods of analysis for whey and whey products, and utilization of wheys and modified wheys in animal feeds. A discussion of all of these topics is beyond the scope of this paper; however, WPI has specifically identified a need for procedural improvements in the analytical measurement of lactose, especially as related to lactose purity. It would be desirable to have methodology available to distinguish a 99 percent pure lactose from a <99 percent lactose product with at least 95 and preferably 99 percent confidence.

Methods (or variations thereof) commonly used for lactose analysis include colorimetric procedures (13, 14), high pressure liquid chromatography (15, 16), gas chromatography (17), enzymatic hydrolysis with β -galactosidase followed by measurement of glucose (18), gravimetric or colorimetric procedures depending on the precipitation of cuprous oxide (19) and polarimetric -procedures (20). The first four are being used by industry at present. The official AOAC procedures, either the Lane-Eynon general volumetric method that incorporates the Soxhlet modifications of Fehling's solution to precipitate cuprous oxide or the Munson-Walker procedure, in which reduced copper is either weighed directly or titrated do not appear to be commonly used. All the colorimetric procedures, although very sensitive, have the disadvantage that all reducing sugars present are measured. Gas chromatography (GC) is considered to be more sensitive than high pressure liquid chromatography (HPLC) for lactose determination by ERRC experts but there may be incomplete derivatization before analysis. The advantage of HPLC is that the derivatization step is eliminated; however, both GC and HPLC methods depend on integration of the area under the curve for quantitation, and reproducibility is usually only about ±1.5 percent, even in the hands of a skilled operator. The enzymatic method depends on complete hydrolysis of lactose. Polarimetry suffers from the disadvantage that other contaminating substances that rotate, such as L-amino acids and other sugars, will influence the measurement.

In spite of this last difficulty, some preliminary experiments have been conducted with polarimetry as the means of detecting differences in lactose purity by the specific rotation α . Measurements were made in a thermostatted tube by reading the angular rotation α at 589 nanometers and then calculating specific rotation α . A Perkin Elmer Model 141 automatic polarimeter was used; this instrument measures angular rotation with an accuracy of \pm 0.002.

The angular and specific rotations obtained when "pure" lactose was contaminated with sodium chloride are listed in Table VI. At an apparent concentration of 5 percent lactose, the difference between angular rotation readings for "100 percent" lactose and "99 percent" lactose is only 0.006, so contamination of less than 1 percent cannot be reliably detected. At lower apparent concentrations, the difference in angular rotation is even less. However, at an apparent concentration of 10 percent lactose, the difference in angular rotation is somewhat greater. With still higher concentrations, it might be possible to detect differences below 1 percent with accuracy. Further work is required to determine what effects contamination with free amino acids or calcium salts would have on the equilibrium rotation values.

TABLE VI.--Specific rotation α at 25 C of lactose contaminated with sodium chloride

NaCl Concentration %	α	α _s .
	Apparent conce	entration 5.00%
0	2.735	+55.14°
1	2.729	+54.58°
	Apparent conc	entration 10.0%
0	5.516	+55.16°
1 · · · · · · · · · · · · · · · · · · ·	5.476	+54.76°

Preliminary experiments suggest that differential scanning calorimetry may offer a sophisticated means of detecting contaminants in lactose because the shape of the melting curve is altered. An isotope ratio method might also be developed; such a procedure would be relatively simple but expensive.

DETECTION OF CASEIN AND WHEY IN FROZEN DESSERTS

Researchers at ERRC have developed a procedure for the detection of whey or non-milk proteins as ingredient substitutes in ice cream and other frozen desserts (21). A collaborative study of the procedure is presently underway to determine its suitability for regulatory purposes.

A simple measurement of the casein to non-casein ratio in the dessert will not suffice because whey proteins will complex with the casein during certain heat treatments (22). Upon subsequent isoelectric precipitation of the casein, the complexed whey protein precipitates with it. Therefore, for determinations of the amount of whey protein in a frozen dessert, the complex

must be broken, or advantage must be taken of some unique property of the protein so as to distinguish them.

Of all the milk proteins, only casein contains phosphorus. The amount of whey protein complexed with the casein is immaterial because the phosphorus content is used to calculate the amount of casein present, once the casein has been precipitated and washed free of contaminating materials. The total nitrogen content also must be measured; once this is known, the amount of whey protein or non-milk proteins present must be calculated by difference. Whey protein of the procedure may be obtained by contacting F. W. Douglas at ERRC.

Additional flexibility was built into the method by use of radial immunodiffusion. Samples may be assayed specifically for casein, whey protein, or a suspected adulterant. Correlation of casein content between the measurement of phosphorus and radial immunodiffusion for several dairy foods is excellent (Table VII), except for the values for chocolate ice cream. Apparently something in the chocolate interferes with the radial immunodiffusion.

TABLE VII. -- Comparison of casein content in some dairy foods measured by phosphorus content and radial immunodiffusion

	% Casein		
Product	RID	Phosphorus	
Coffee whitener	2.63	2.98	
Dry milk	25.85	26.56	
Vanilla ice cream	2.50	2.33	
Vanilla ice cream	3.27	3.51	
Vanilla ice milk	2.41	2.98	
Chocolate ice cream	3.46	2.15	
Strawberry ice cream	1.94	2.07	

OTHER RESEARCH PRIORITIES FOR THE 1980'S

Several areas needing additional research were pinpointed during the Second International Whey Research Workshop held in New Zealand in April 1979. These included ultrafiltration and reverse osmosis, demineralization, functionality, modification of whey and whey components, fermentation, lactose crystallization and hydrolysis, and analytical methodology (23).

All the research priorities listed are discussed in the conference proceedings (23). However, some discussion of needed analytical methodology research is appropriate here because some ERRC research personnel are involved with problems related to these.

First, methods need to be evaluated further for the determination of free and combined water in products with significant lactose content, which FSQS has considered to be a problem. ERRC researchers have cooperated with FSQS on this in the past.

Second, standard methods are needed to determine the total lipid and the nature of the lipid remaining in whey protein concentrates, because residual lipid can seriously affect functionality and storage stability.

Third, a study of the various methods available for quantifying whey protein denaturation should be undertaken. At present, ERRC personnel are collaborating with researchers at a number of universities in a study of methods for measuring functionality. The first property selected for study was protein solubility.

Fourth, because of the limitations of the protein efficiency ratio (PER) method, additional work is needed to standardize methods for nutritional value measurement. Researchers at ERRC are presently participating in a collaborative study sponsored by the American Society of Testing Materials and the American Association of Cereal Chemists.

Fifth, additional methods need to be developed for estimating ionic calcium.

Sixth, better methods for defining and estimating the various fractions of whey protein are needed to determine true protein concentration.

Seventh, new methods of evaluating protein interactions are needed. Such interactions involve protein-protein, protein-mineral, protein-lipid and protein-mineral-lipid interactions. These interactions are all related to protein denaturation and the final functionality of the protein. At ERRC, some fundamental studies of protein-lipid interactions are being conducted. One approach has been to examine protein conformational changes brought about when interaction occurs upon spreading the protein out in a thin film over a lipid-containing medium.

Eighth, refinement of methods for determination of lactose and its hydrolyzed products is needed for reasons that have already been described.

CONCLUSIONS

Much of the international whey research of the 1980's will probably be directed toward solving many of the problems described here. At ERRC, at present, dairy research as a whole has been greatly reduced; however, ERRC research scientists are always available for consultation on industrial problems.

ACKNOWLEDGMENTS

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Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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